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Molecular Characterisation of Type 1 Polioviruses Associated With Epidemics in South Africa

Claudia Chezzi,* Nigel K. Blackburn, and Barry D. Schoub

National Institute for Virology and Department of Virology, University of the Witwatersrand Medical School, Johannesburg, South Africa

The molecular epidemiology of wild-type 1 polioviruses isolated in South Africa during 2 major poliomyelitis epidemics in the 1980s and during the pre- and inter-epidemic periods was investigated by partial sequence analysis across the VP1/2A junction. Poliovirus-specific primers were used to amplify and subsequently sequence the region of interest. Viruses belonging to different genotypes were found to have been responsible for the 2 outbreaks. The Gazankulu outbreak in 1982 was caused by a poliovirus genotype which was unique to South Africa and which circulated endemically throughout much of the country between 1980 and 1985. Two additional genotypes, imported from the Middle East and West Africa, cocirculated endemically with the South African genotype between 1982 and 1985. The 1988 epidemic in Kwazulu-Natal was attributed to an imported genotype apparently introduced into South Africa in 1985 from countries north of the border. This genotype displaced the 3 genotypes previously in circulation and continued to be transmitted within the country until 1989, when the last confirmed cases of poliomyelitis associated with wild-type viruses were documented. All circulating wild-type poliovirus strains appear to have been eliminated from South Africa. ***J. Med. Virol. 52:42–49, 1997.***

KEY WORDS: poliomyelitis; epidemiology; partial sequence analysis

INTRODUCTION

In 1988 the World Health Organization (WHO) set a target for the global eradication of poliomyelitis by the year 2000 [1988]. Despite intensive efforts toward the achievement of this goal, in developing countries, particularly in Asia and Africa, poliovirus-induced paralytic poliomyelitis continues to remain an important cause of debility and death [WHO, 1995].

Two extensive epidemics of poliomyelitis occurred in South Africa during the 1980s. In 1982, an outbreak lasting from May to September, during which 260 cases of paralytic polio and 42 deaths were reported, took place in Gazankulu in the northeastern part of the country [Johnson et al., 1984; Saayman et al., 1984]. This was followed in 1987 and 1988 by an even more extensive outbreak, involving 412 paralytic cases and 42 deaths, in Kwazulu-Natal along the southeastern coast of the country [Schoub et al., 1992; Van Middelkoop et al., 1992]. Type 1 polioviruses were found to be responsible for both outbreaks, which, however, differed markedly from each other with respect to population immunity [Schoub et al., 1992]. The Gazankulu outbreak was characterised by a low level of population immunity, with 74% of patients in the epidemic exhibiting no detectable antibodies to polio types 2 and 3, while exhibiting high antibody titres to the causative type 1 virus [Johnson et al., 1984]. Surveillance carried out shortly after the epidemic revealed substantial underimmunization, with vaccine coverage as low as 43% in some rural areas [Johnson et al., 1984]. Reduced vaccine potency, probably due to breaks in the cold chain, also contributed to the vaccine failure in the area. In contrast, the Kwazulu-Natal epidemic occurred under conditions of high immunity, with antibodies to all 3 polio serotypes reported for 76% of children under the age of 2 in the area [Schoub et al., 1992]. Severe flooding during the previous year caused a disruption in basic health services and sanitation, leading to the build-up of a massive viral burden in the area [Van Middelkoop et al., 1992]. The large amount of circulating virus was able to spread rapidly among the relatively small population of susceptibles in the community, resulting in an extensive epidemic.

The molecular epidemiology of both outbreaks has been investigated previously by oligonucleotide fingerprint analysis. Using this technique it was found that the molecular epidemiological characteristics of the 2 outbreaks were different; during the Gazankulu epidemic, several different unrelated strains of viruses were found to be in circulation [Tsilimigras et al.,

*Correspondence to: Claudia Chezzi, National Institute for Virology, Private Bag X4, Sandringham, 2131, South Africa.

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1984], whereas a single strain of wild-type polio 1, also isolated from other areas of South Africa, was implicated in the Kwazulu-Natal outbreak [Tsilimigras et al., 1991]. Oligonucleotide fingerprinting has been used extensively in molecular epidemiology studies of poliovirus [Kew and Nottay, 1984]; however, the technique is very sensitive to small mutational changes and reliable estimates of relatedness can be made only when viral strains show >95% sequence homology [Rico-Hesse et al., 1987]. Polioviruses evolve at a rate of approximately 1–2 base substitutions over the entire genome per week, or 1–2% per year [Nottay et al., 1981], and fingerprinting is thus limited to recognising relationships between isolates separated by no more than 3–5 years. An investigation of the epidemiological interrelationships between isolates from the Gazankulu and Kwazulu-Natal epidemics and isolates from different parts of the world thus required the use of an alternative technique. Partial genomic sequencing of 150 bases across the VP1/2A region of the poliovirus genome has been found to be the most definitive technique for the determination of genetic relationships between polioviruses [Rico-Hesse et al., 1987]. It has been found that wild-type polioviruses of all 3 serotypes tend to cluster geographically, and that analysis of the extent of sequence divergence between poliovirus strains can lead to the identification of epidemiological links that cannot be established by any other means [Rico-Hesse et al., 1987; Kew et al., 1990]. In this study we therefore investigated, using partial sequence analysis, the molecular epidemiology of polio 1 strains isolated in South Africa during the 1982 and 1987–1988 epidemics and during the interepidemic period and describe a rapid method for differentiating between isolates from the two outbreaks by restriction fragment length polymorphism (RFLP).

MATERIALS AND METHODS

Viruses

Eighteen poliovirus type 1 strains obtained during the 1982 Gazankulu outbreak and 20 strains from the 1987–1988 Kwazulu-Natal outbreak were originally isolated from clinical specimens of patients suffering from poliomyelitis as described previously [Johnson et al., 1984; Schoub et al., 1992]. An additional 82 non-epidemic strains, isolated from patients with a clinical diagnosis of poliomyelitis during the period 1980–1989, were also investigated. The Sabin 1 poliovaccine strain (P1/Lsc2ab), obtained from the Vaccine Unit, NIV, was used as the reference strain for alignments and comparisons. Intratypic differentiation between vaccine-like and wild-type strains was performed using the PCR assay of Yang et al. [1991].

Viral RNA Sequencing

Initially sequencing was performed on purified viral RNA as described by Rico-Hesse et al. [1987]. To facilitate sequencing, viral RNA was amplified using the polymerase chain reaction (PCR) and the PCR products sequenced directly. All sequences that were initially obtained directly from RNA templates were subsequently confirmed by sequencing of PCR templates.

Primers

In order to generate templates for sequencing, a 293 base pair (bp) fragment encompassing the 150 bp region used for genotype analysis was amplified using poliovirus-specific primers. Primer 2A (5′ AAG AGG TCT CTA TTC CAC AT), previously described by Rico-Hesse et al. [1987], was used as the reverse primer. Forward primer PVPCR2 (5′ GTC AAT GAT CAC AAC CCC AC) was chosen from a conserved region of the poliovirus VP1 region after analysis of published Sabin and wild-type sequences [Chezzi, 1996].

Virus RNA Extraction for PCR

RNA was extracted from 160 μl of infected tissue culture fluid. Forty microlitres of 5X lysis buffer (250 mM Tris-HCl pH 8.3, 350 mM KCl, 25 mM $MgCl_2$, 2.5% NP-40) were added to each tube, and tubes were incubated on ice for 15 minutes. Nucleic acids were extracted once with phenol, once with phenol/chloroform (1:1), and once with chloroform/isoamyl alcohol (24:1). The aqueous supernatants were used as templates for PCR reactions and subsequently stored at −70°C.

Reverse Transcription and PCR

Reverse transcription and PCR (RT-PCR) were performed in a single step as previously described [Chezzi, 1996]. Briefly, reactions were carried out in 100 μl volumes using the following conditions: 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM of each deoxynucleoside triphosphate, 10 units RNase inhibitor (Boehringer Mannheim), 5 units AMV-reverse transcriptase (Boehringer Mannheim), 2.5 units Taq polymerase (Boehringer Mannheim), and 20 pmol of each primer. Thermal cycling for RT-PCR was performed on a Biometra Trioblock using the following programme: 1 cycle of reverse transcription (42°C, 45 minutes); 1 cycle of denaturation (95°C, 3 minutes); 30 cycles of denaturation (95°C, 30 seconds), annealing (52°C, 45 seconds), and elongation (72°C, 1 minute); and one final cycle of elongation (72°C, 7 minutes). Amplified products were analysed by electrophoresis through 2.5% agarose gels containing 0.4 μg/ml ethidium bromide.

Sequencing of Amplified Products

PCR products were purified from agarose gels using the Mermaid kit (Bio101). Briefly, 40 μl of amplified product were electrophoresed on a 1.5% low-gelling temperature Biogel (Bio101) in Mermaid buffer, and the target bands excised from the gel. DNA was purified from the agarose slices according to the manufacturers' instructions and eluted in a final volume of 20 μl distilled water. Five microlitres of purified DNA was used for sequencing. Dideoxy sequencing of purified PCR products was performed using the Sequenase PCR-product sequencing kit (United States Biochemicals) with ^{35}S-dATP and primer 2A as the sequencing

TABLE I. Predicted Sizes of Fragments Produced by RsaI Cleavage of the 293 bp PVPCR2/2A Fragment

Strain	Fragment size (bp)			
Sabin 1	103	91	67	32
Sabin 2	153	91	46	
Sabin 3	101	91	65	33
Wild-type 1—genotype I	155	138		
Wild-type 1—genotype III	202	91		

primer. All sequence data obtained was confirmed by repeat sequencing with primer PVPCR2. Sequencing products were resolved on 8% acrylamide gels containing 7M urea and visualized by autoradiography. Sequence data was analysed using DNASIS software (Hitachi). Genetic relationships between poliovirus isolates were determined by the method of least squares using the KITSCH programme from the PHYLIP phylogenetic inference package [Felsenstein, 1993]. A genotype was defined as a group of viruses showing at least 85% nucleotide sequence similarity across the 150 bp VPI/2A region, and within genotypes, direct epidemiological links were defined by 98% or greater sequence homology between strains [Rico-Hesse et al., 1987]. Sequences for the following isolates were kindly made available to us by O. M. Kew, CDC: 6224/ZIM85; 1197/JOR78; 1177/KUW77; 6747/SEN86.

Restriction Enzyme Analysis

The 150 bp sequences of representative isolates belonging to genotypes I and III were scanned for potential restriction sites that would enable us to rapidly assign isolates to the respective genotypes by RFLP analysis of the 293 bp PVPCR2/2A product without resorting to sequencing. Ideally we wanted to find a single enzyme that would produce different restriction patterns for genotype I and III sequences, respectively. Although only 150 bp of sequence were available for analysis, the sequences flanking the 150 bp region within the 290 bp product are relatively well conserved between the 3 Sabin poliovirus serotypes [Toyoda et al., 1984], and it was thus considered unlikely that any mutations conferring a restriction enzyme site characteristic of genotype would occur in those regions. Of the enzymes that cut within the 150 bp region, only RsaI was predicted to produce an RFLP pattern that could distinguish between the 2 genotypes, as well as between wild-type and vaccine-like type 1 viruses (Table I), and this enzyme was therefore used for RFLP analysis. Ten μl of amplified PVPCR2/2A PCR products were digested with restriction enzyme RsaI (Boehringer Mannheim) in a total reaction volume of 20 μl. Incubation was at 37°C for 1 hour in the appropriate buffer. Digests were analysed on 10% polyacrylamide gels.

RESULTS

Wild-type polioviruses isolated during the 1982 and 1987–1988 epidemics in South Africa were characterised by partial genomic sequencing of 150 bp across the VP1/2A junction. Strains obtained during the pre- and post-epidemic years were also included in the analysis. The relationships between South African type 1 poliovirus isolates is represented graphically in Figure 1. The sequences of representative isolates only have been included in the dendrogram, and results for strains that are closely related are not presented. All polioviruses investigated fell within 4 distinct genotypes, I–IV, defined by a shared nucleotide identity of 85% or more within the 150 bp region analysed. Three genotypes were co-circulating in South Africa between 1980 and 1985; the fourth genotype was introduced into the country in 1985 and was the sole genotype in circulation until 1989. All poliovirus strains isolated in the country since 1989 have been vaccine-like.

Analysis of the relationships between wild-type polioviruses obtained during the 2 epidemics indicated that the outbreaks were caused by viruses belonging to different genotypes. All strains associated with the 1982 Gazankulu epidemic fell within genotype I. Epidemic isolates from the Gazankulu area (1325/SOA82; 1532/SOA82; 4238/SOA82) and from cases seen in neighbouring hospitals during the outbreak period (Pietersburg, 1677/SOA82) were very closely related (maximum divergence between them, 3%) and formed a separate cluster within genotype I. In addition to the epidemic isolates, strains belonging to genotype I were isolated between 1980 and 1985 from the Johannesburg area (698/SOA83; 1897/SOA80), the Free State (1170/SOA83), Kwazulu/Natal (1897/SOA80; 5917/SOA82), and the Eastern Cape (282/SOA85). A high degree of diversity (8–11%) between strains belonging to the same genotype was evident. Sequence divergence between the epidemic strains and other strains within the same genotype was approximately 7%. Comparison of genotype I viruses to a databank of strains obtained worldwide revealed no relationship with known strains from other countries, making this genotype unique to South Africa.

The 1987–1988 Kwazulu-Natal epidemic was associated with viruses belonging to genotype III. The majority of the epidemic isolates (359/SOA88; 221/SOA88; 364/SOA88) were very closely related (maximum sequence divergence, 2%). Cases with definitive epidemiological links to the Kwazulu-Natal epidemic (less than 2% sequence divergence) were also seen in 1988 in Johannesburg (1522/SOA88; 427/SOA88) and Port Elizabeth, on the southeastern coast of the country (1048/SOA88). Several epidemic strains (181/SOA88) and strains isolated in Johannesburg during the same period (402/SOA88; 400/SOA88) formed a separate cluster diverging from the majority of the outbreak isolates by 4%, although within this group isolates from Kwazulu-Natal (181/SOA88) displayed only 2% divergence from the epidemic strains.

Polioviruses belonging to genotype III were first isolated in the former province of Transvaal in the north of South Africa in 1985. The closest relative to the genotype III strains was a 1985 strain from Zimbabwe (6224/ZIM85). By early 1986, strains belonging to this genotype were isolated throughout the former province

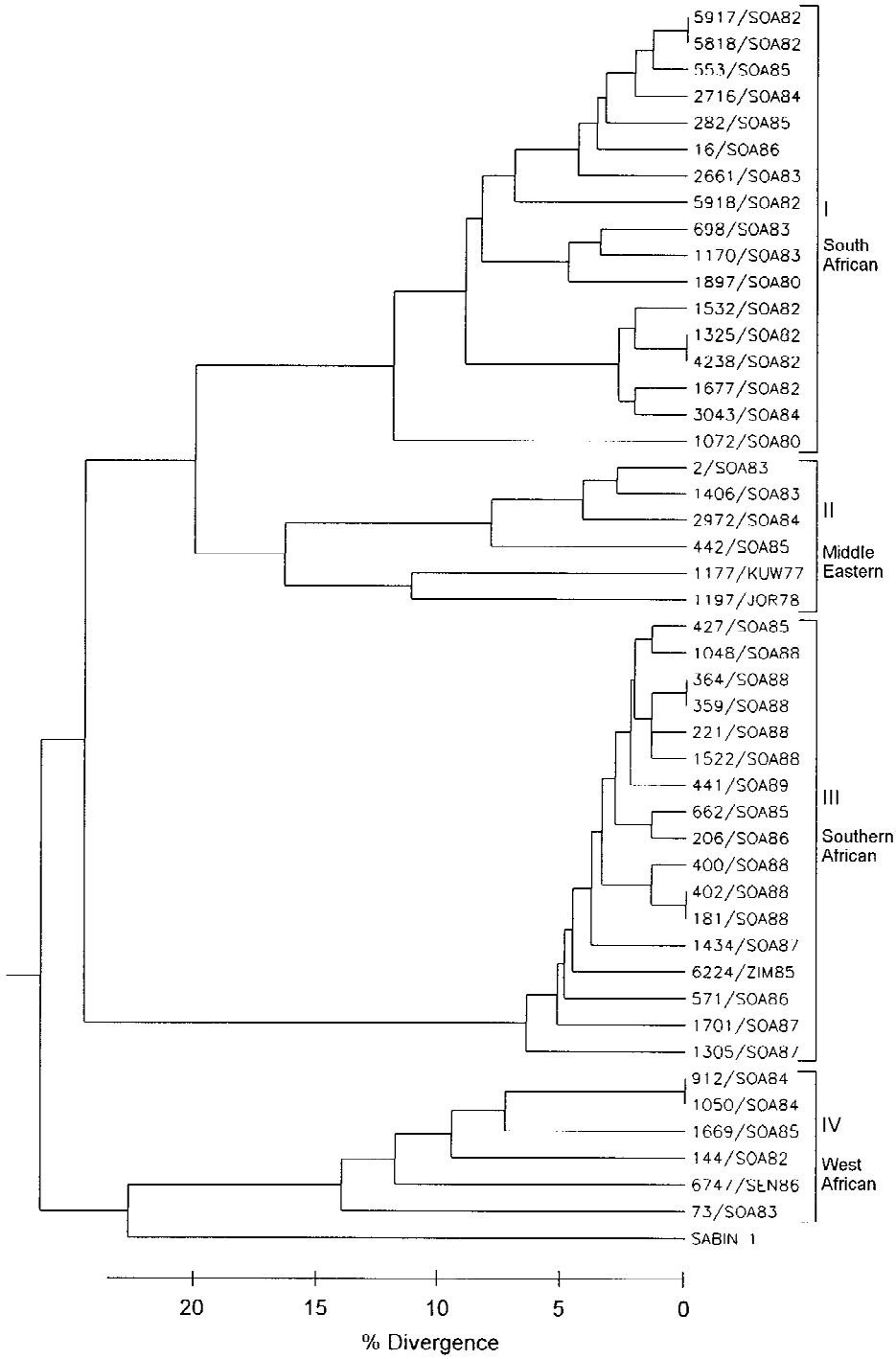

Fig. 1. Dendrogram of sequence relationships between South African poliovirus isolates. Genotypes are grouped with parentheses and assigned numbers (I–IV). The extent of sequence divergence between any 2 strains is determined by measuring the distance along the X-axis to the connecting node. Note that the distance between all sequences connected by the same node represents average divergence. The following country abbreviations are used: JOR, Jordan; KUW, Kuwait; SEN, Senegal; SOA, South Africa; ZIM, Zimbabwe.

of Transvaal (206/SOA86), and isolation of strain 571/SOA86 from a case in Durban in March 1986 indicated that genotype III had spread into Kwazulu-Natal as well. Viruses isolated throughout the country between 1985 and the end of 1987 displayed a relatively high degree of sequence divergence (5%–6%). The last circulating strain belonging to the genotype III, 441/SOA89, isolated in Johannesburg in 1989, exhibited 97% sequence similarity to the Natal epidemic strains and 96% similarity to the 1988 isolates from Johannesburg.

Genotypes I and III could be readily distinguished by analysis of the RFLP patterns generated by restriction

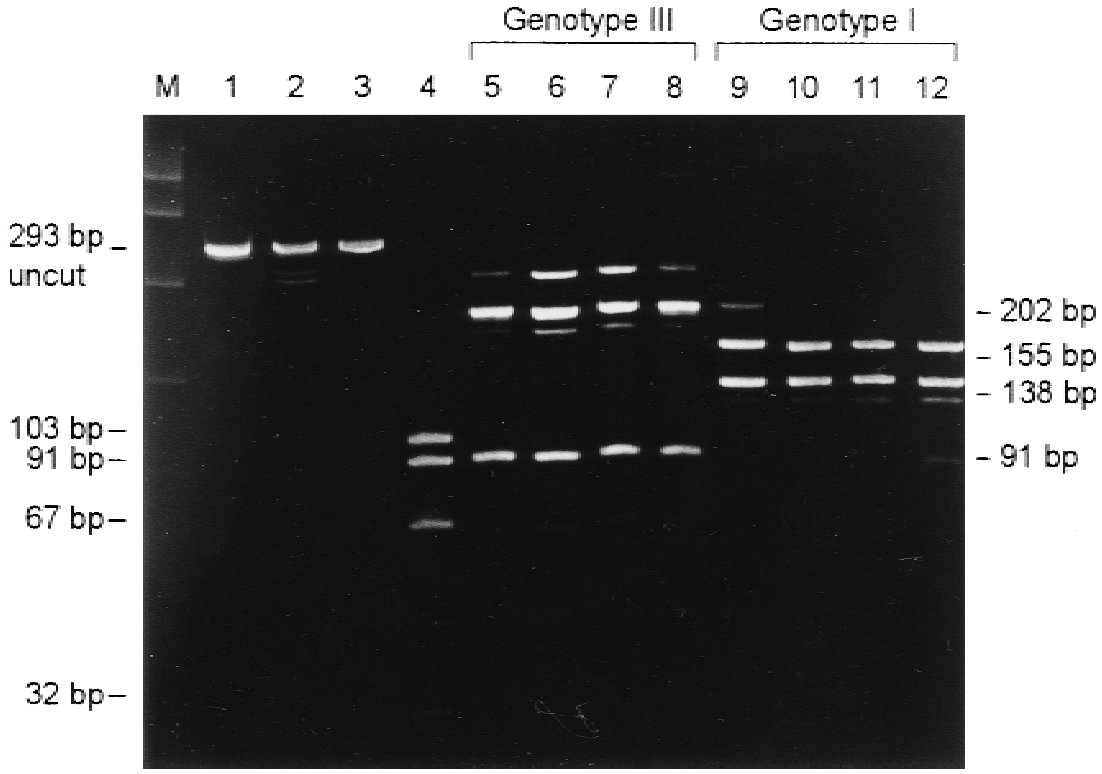

Fig. 2. RFLP patterns produced by RsaI cleavage of South African poliovirus isolates belonging to genotype I and III. Lane M, 0X174/TaqI molecular weight markers; lane 1, Sabin 1, uncleaved; lane 2, 359/SOA88, uncleaved; lane 3, 1325/SOA82, uncleaved; lane 4, Sabin 1; lane 5, 181/SOA88; lane 6, 402/SOA88; lane 7, 359/SOA88; lane 8, 1701/SOA87; lane 9, 1325/SOA82; lane 10, 1677/SOA82; lane 11, 4238/SOA82; lane 12, 5917/SOA82.

of the 293 bp PCR product with RsaI (Fig. 2). The sizes of the fragments obtained corresponded exactly to those expected (Table I), indicating that no additional cleavage sites were present upstream or downstream of the 150 bp VP1/2A junction region within the 293 bp amplicon. Strains which diverged by as much as 6% within genotype I (5917/SOA82 and 1325/SOA82) and 4% within genotype III (1701/SOA87 and 359 SOA88) produced identical RFLP patterns. However, strains within genotype III which diverged from the Kwazulu-Natal epidemic strains by more than 5% contained an additional cleavage site within the 150 bp region, leading to the production of fragments of 135 bp, 91 bp and 67 bp (results not shown). In some cases, additional faint background bands, corresponding to undigested nonspecific amplification products, could also be seen.

Two additional poliovirus genotypes, II and IV, were also in circulation in South Africa between 1982 and 1985. Strains belonging to genotype II were circulating in the former province of Transvaal between 1983 and 1985. The minimum divergence between these strains was 3% (between 2/SOA83 and 140/SOA83): later isolates from 1984 (2972/SOA84) and 1985 (442/SOA85) exhibited 94% similarity to the 1983 isolates. The closest relatives to these strains (85–88% similarity) were older strains from Jordan (1197/JOR78) and Kuwait (1177/KUW77). Genotype IV comprised 5 isolates, obtained from geographically distant areas in the former province of Transvaal, the Free State, and Kwazulu-Natal. Sequence divergence between the isolates reached 11%. Comparison of these strains to the sequence databank showed them to be approximately 87% similar to strains from West Africa (6747/SEN86).

DISCUSSION

Partial genomic sequence analysis of 150 bp across the VP1/2A region was employed to characterise and study the molecular epidemiology of wild-type polioviruses associated with cases and outbreaks of poliomyelitis in South Africa in the past decade. It has been shown that analysis of this region enables one to determine the patterns of circulation of wild-type polioviruses and can provide information on epidemiological links between cases and outbreaks [Rico-Hesse et al., 1987]. This approach has been used successfully to study the worldwide distribution of the 3 serotypes of polioviruses [Huovilainen et al., 1995; Lipskaya et al., 1995; Mulders et al., 1995; Zheng et al., 1993; Rico-Hesse et al., 1987].

Four type 1 genotypes were found to have been present in South Africa during the 1980s. Three independent genotypes, I, II, and IV, co-circulated in the country between 1980 and 1985. The majority of isolates from this period fell into genotype I, which was unique to South Africa and circulated endemically throughout the country until its displacement in 1986. Strains from genotype I were implicated in the 1982 Ga-

zankulu outbreak [Saayman et al., 1984]. Previous molecular epidemiological studies, which used oligonucleotide fingerprint analysis to determine relationships between strains, suggested that the outbreak was multifocal, arising from several unrelated poliovirus strains present in the area at the same time [Tsilimigras et al., 1989]. Our sequence analysis results, however, indicate that the epidemic isolates from the Gazankulu area were highly homologous and diverged by a maximum of 2%. Isolates obtained from more outlying hospitals exhibited slightly less relatedness to the outbreak strains (3% divergence). The high degree of sequence relatedness between outbreak isolates is consistent with rapid epidemic spread of a single strain and is thus indicative of a single source epidemic. Oligonucleotide fingerprinting is highly sensitive to mutations and reliable estimates of relatedness can only be made when strains share greater than 95% sequence similarity. All outbreak strains showed less than 5% sequence divergence within the interval sequenced, and as such, would have been expected to present similar oligonucleotide patterns. However, when the nucleotide differences between strains are very small, discrepancies between fingerprinting and sequencing results may arise [Rico-Hesse et al., 1987]; oligonucleotide fingerprinting samples a much greater portion of the genome (10–15%), thus increasing the genomic interval used for comparison, so that limited sequence data may underestimate the differences between very closely related viruses. The 2% divergence between epidemic strains isolated over a 6-month period is suggestive of rapid virus transmission, and it is thus possible that these viruses, despite displaying a high degree of sequence similarity across a limited genomic interval, might have produced slightly different oligonucleotide fingerprint patterns. More detailed sequence analysis of a larger portion of the genome would be required in order to reveal more complex relationships between isolates. The unavailability of strains isolated in the Gazankulu area before the epidemic does not allow us to unequivocally determine whether the epidemic strain was already present in the area before the outbreak or whether it was introduced from elsewhere. Genotype I strains were certainly in circulation in the former province of Transvaal as early as 1980 (1072/SOA80), and the high degree of divergence between strains within genotype I (up to 11%) indicates that several pockets of susceptible individuals, sustaining independent evolution of the same genotype, existed in the country. However, the absence of any close epidemiological links (<2% sequence divergence) between strains circulating in other areas of the country during or shortly after the epidemic (5917/SOA82, Kwazulu-Natal and 698/SOA83, Johannesburg) would suggest that the epidemic strain was already circulating in the Gazankulu area prior to the outbreak.

The extensive 1987–1988 outbreak in Kwazulu-Natal was attributed to strains belonging to genotype III. The 95–97% sequence similarity between epidemic isolates and strains isolated in the area in the preceding years (571/SOA86; 1701/SOA87) indicates that the epidemic was caused by strains that were already in circulation in the area and not by the introduction of a novel genotype into a susceptible population. Sequence analysis of the isolates associated with the outbreak showed them to be very closely related, consistent with rapid epidemic spread of a single strain. Strains very closely related to the epidemic isolates were also isolated around the Johannesburg area during the outbreak; travel between the rural and semi-rural areas of the country to the large cities is a common feature of life in South Africa, and the presence of epidemic strains in the former province of Transvaal is most likely the result of patients from Kwazulu-Natal traveling to and seeking care in hospitals in the Johannesburg area. Very good concordance was seen between the sequencing results reported in this study and earlier oligonucleotide fingerprint analysis of strains associated with the outbreak, which implicated a single strain, which was also in circulation in other parts of the country during the epidemic [Tsilimigras et al., 1991].

The absence of strains belonging to genotype III in South Africa prior to 1985, and evidence of genetic relatedness to a 1985 isolate from Zimbabwe strongly suggests that genotype III was introduced into South Africa from countries north of the border. The earliest isolates belonging to genotype III were made in the former Transvaal province; a likely route of importation into South Africa may have been across the border with Zimbabwe or Mozambique, via refugee movements due to political and social unrest. The isolation of strains related to those from the former Transvaal approximately 1 year later in Kwazulu-Natal suggests that the transmission route was from the former Transvaal into the other provinces. The relatively high degree of sequence divergence between strains isolated during the 3-year period between 1985 and the end of 1987 is suggestive of rapid transmission among susceptible individuals.

Genotype III displaced the locally circulating genotypes I, II, and IV and continued to circulate until 1989, when the last confirmed cases of poliomyelitis attributed to wild-type viruses were documented [Schoub et al., 1995]. Displacement of endemically circulating strains by an imported strain has been shown to be favoured by conditions of inadequate population immunity, thus allowing the introduction and sustained transmission of an imported strain amongst susceptible individuals [Rico-Hesse et al., 1987]. Vaccine coverage in South Africa dropped from 76–77% during 1984 and 1985 to 70% in 1986 (Department of National Health, 1994), providing the conditions of low population immunity required for the establishment of endemicity by the imported genotype III. Displacement of endemic genotypes has also been reported from other developing countries, including Venezuela and Honduras [Rico-Hesse et al., 1987] and the Central African Republic [Morvan et al., 1996].

Genotypes II and IV represent genotypes imported

from countries in the Middle East and West Africa, respectively. Although the number of isolates belonging to the latter 2 genotypes is small, the high degree of genetic diversity between isolates is suggestive of rapid establishment of endemicity, in separate pockets of susceptible populations, by progenitor infections in the late 1970s.

The RFLP assay was developed in order for isolates to be rapidly assigned to the 2 major genotypes in circulation in South Africa and was successfully employed for the initial screening of isolates obtained during the pre- and post-epidemic years. RFLP analysis in the study of poliovirus genomic variability has also been described by Balanant et al. [1991] and has successfully been employed to study the molecular epidemiology of polioviruses isolated in Israel [Vonsover et al., 1993] and in the Central African Republic [Morvan et al., 1996]. The RFLP assay described here can be modified for the characterization of additional genotypes simply by screening for restriction sites specific for the genotype under analysis. For example, the identification of a unique DdeI site in isolates from the 1993 Nambian outbreak [Van Niekerk et al., 1994] allowed us to rapidly screen epidemic isolates prior to sequencing (results not shown). Even within genotypes I and III, mutations leading to the gain of alternative cleavage sites specific for each genotype, such as a unique SmaI site for genotype III isolates, were noted. However, we chose to use RsaI as it allowed us to differentiate between genotypes I and III and between wild-type and Sabin 1–like viruses in a single reaction.

Because the RFLP assay described in this study is based on the analysis of the region spanning the VP1/2A junction which was also employed for the identification of relationships based on sequence data, relationships inferred from the RFLP patterns can be directly compared to those observed from sequence data. It must be noted, however, that our RFLP assay was not designed for fine resolution studies of wild-type poliovirus circulation, and as such, it is not able to provide detailed insights into the relationships between wild-type strains. Nevertheless, it provides a means for rapid screening of large number of isolates from a single country, and is most effective when the number of circulating genotypes within a country are limited to 3 or less. The conservation of the cleavage site within a genotype, even between strains displaying up to 6% divergence, and the ease of performance of the assay make the RFLP approach more practical than that of genotype-specific PCR for screening for individual genotypes [Yang et al., 1992].

In conclusion, we have described the molecular epidemiology of wild-type 1 polioviruses associated with 2 major outbreaks of poliomyelitis in South Africa. The 1982 Gazankulu epidemic was associated with genotype I, which was unique to South Africa and had been in circulation since at least 1980. Two additional imported genotypes circulated concurrently with genotype I until 1985. The 1987–1988 Kwazulu-Natal outbreak was caused by a different genotype, introduced into South Africa several years earlier from countries north of the border. The displacement of the 3 locally circulating genotypes by the imported genotype reflects the transition from endemic to epidemic poliovirus circulation as vaccine coverage in South Africa increased. No wild-type viruses have been isolated since 1989, and it thus appears that the transmission of wild-type polioviruses in South Africa has been effectively controlled.

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